Psychopharmacology (2001) 157:327–339
DOI 10.1007/s002130100859

REVIEW

A. Shekhar · U. D. McCann · M. J. Meaney
D. C. Blanchard · M. Davis · K. A. Frey · I. Liberzon
K. L. Overall · M. K. Shear · L. H. Tecott · L. Winsky

Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders

Received: 27 March 2001 / Accepted: 6 June 2001 / Published online: 18 August 2001

Abstract *Rationale:* There exists a wide range of animal models and measures designed to assess anxiety or fearfulness. However, the relationship between these models and clinical anxiety symptoms and syndromes is unclear. The National Institute of Mental Health convened a workshop to discuss the relationship between existing behavioral models of anxiety and the clinical profile of anxiety disorders. A second goal of this workshop was to outline various approaches towards modeling components of anxiety disorders. *Objectives:* To briefly describe epidemiological and behavioral manifestations of clinical anxiety syndromes and how they relate to commonly employed animal models of anxiety. To describe approaches and considerations for developing, improving, and adapting anxiety models to better understand the neurobiology of anxiety. *Methods:* Clinicians, psychiatrists and clinical and basic neuroscientists presented data exemplifying different approaches towards understanding anxiety and the role of animal models. Panel members outlined what they considered to be critical issues in developing and employing animal models of anxiety. *Results:* This review summarizes the discussions and conclusions of the workshop including recommendations for improving upon existing models and strategies for developing novel models. *Conclusions:* The probability of developing comprehensive animal models that accurately reflect the relative influences of factors contributing to anxiety disorder syndromes is quite low. However, ample opportunity remains to better define and extend existing models and behavioral measures related to specific processes that may be disrupted in anxiety disorders and to develop new models that consider the impact of combined factors in determining anxious behaviors.

Keywords Stress · Panic · Obsessive-compulsive disorder · PTSD · Startle · Maternal grooming · Mutant mice · Ethological models · Benzodiazepine

A. Shekhar
Indiana University, Indiana, USA

U.D. McCann
Johns Hopkins University, Baltimore, Maryland, USA

M.J. Meaney
McGill University, Montreal, Canada

D.C. Blanchard
University of Hawaii, Hawaii, USA

M. Davis
Emory University, Atlanta, Georgia, USA

K.A. Frey · I. Liberzon
University of Michigan and Ann Arbor VAMC, USA

K.L. Overall
University of Pennsylvania, Pennsylvania, USA

M.K. Shear
University of Pittsburgh, Pittsburgh, Pennsylvania, USA

L.H. Tecott
University of California, San Francisco, California, USA

L. Winsky (✉)
National Institute of Mental Health,
6001 Executive Blvd MSC 9641, Bethesda, MD 20892-9641, USA
e-mail: lois@helix.nih.gov
Tel.: +1-301-4435288, Fax: +1-301-4024740

Introduction

Much of our understanding of the neural substrates of anxiety disorders has emerged from studies employing animal models that emulate aspects of the presumed etiology, physiology, or behavioral expression of fear and anxiety. The identification of circuits mediating these behaviors continues to be an essential first step toward understanding the neurobiological mechanisms that contribute to normal and pathological fear and anxiety (Gray et al. 1981; Lister 1990; Willner 1991; Martin 1998). These behavioral models are also important screening tools for identifying drugs with potential anxiolytic action (Barrett and Vanover 1993; Martin 1998). For example, the conflict and social interaction paradigms have been extremely effective in identifying benzodiazepine compounds. These models often had the

Table 1 Some currently utilized tests relevant to anxiety[a]

1. Punishment-induced conflict	Geller-Seifter (1960) Test Vogel Test (Vogel et al. 1971) Conditioned Suppression (Theibot et al. 1980)
2. Ethological conflict	Open Field Test (Hall 1934) Elevated Plus-Maze (Pellow and File 1986) Light-Dark Compartment Test (Costall et al. 1989) Social Interaction Test (File 1980)
3. Aversive tests	Defensive Probe-Burying (Treit et al. 1981) PAG Sstimulation (Audi and Graeff 1984) Exposure to predator (Blanchard et al. 1997)
4. Drug discrimination tests	Pentylenetetrazol administration (Andrews and Stephens 1990) Other anxiogenic drugs (Leidenheimer and Schechter 1988)
5. Conditioned fear tests	Fear-potentiated startle (Davis 1989) Conditioned place-aversion (Cunningham 1981)
6. Developmental models	Maternal separation model (Hofer 1973; Plotsky and Meaney 1993) Neonatal grooming model (Meaney et al. 1988) Neonatal sepsis model (Shanks et al. 1995)
7. Pathophysiological models	Hypothalamus dysfunction model of panic disorder (Shekhar 1994) Amygdala priming model of anxiety (Sajdyk et al. 1999; Shekhar et al. 1999) Trauma and HPA axis sensitization models of PTSD (Liberzon et al. 1999a)
8. Transgenic models	Review (Tarantino and Bucan 2000) Serotonin receptor knockout mice (Heisler et al. 1998; Parks et al. 1998; Ramboz et al. 1998) CRF receptor knockdown models (Heinrichs et al. 1997) GABA system mutant mice (Kash et al. 1999)

[a] References are representative

added advantage of distinguishing drug effects on conflict measures from other simultaneous behaviors (Geller and Seifter 1960; File 1980; Martin 1998). Several of these tests are also sensitive to non-benzodiazepine anxiolytic drugs, although results with serotonergic drugs have been more variable (Barrett and Vanover 1993; Griebel 1995; Hogg 1996). Table 1 provides an annotated listing of some commonly employed manipulations and measures for examining the neurobiology of fear and anxiety. Thorough reviews of data obtained from these models are available elsewhere (Griebel 1995; Martin 1998; Weiss et al. 2000), as are summaries of the factors affecting these indices of putative anxiolytic or anxiogenic activity including species, strain, sex, handling conditions, and testing apparatus (Barrett and Vanover 1993; Griebel 1995; Hogg 1996). These considerations will not be re-examined in the current manuscript. Rather, the focus is on examining the degree to which these measures might relate to specific aspects of anxiety disorders or reflect actions within discrete CNS regions contributing to anxiety.

Current classification of anxiety disorders

Currently, the Diagnostic and Statistical Manual for Psychiatric Disorders – IV edition (DSM-IV; APA 1995) distinguishes several distinct anxiety syndromes each having its own clinical presentation, course, and response to treatment. Therefore, a brief review of the current classification of anxiety disorders was an important starting point for discussing animal models relating to these disorders.

Panic disorder

This disorder is characterized by unexpected panic attacks consisting of brief, sudden, intense episodes of anxiety associated with a variety of physiological symptoms of autonomic activation and psychological feelings of impending harm (APA1995; Fyer et al. 1996). The disorder affects about 2% of the population, usually beginning in teens and early 20s, and is twice as common in women than men (Klerman et al. 1991; Kessler et al. 1994). Phobias (e.g., agoraphobia), fear, and avoidance of places or situations where panic attacks have occurred or where the individual may feel trapped in the event of a panic attack, are commonly associated with panic disorder. Panic disorder is often comorbid with depression and alcohol abuse (Kessler et al. 1997). Patients suffering from this disorder respond well to focused cognitive and behavioral therapies and/or chronic treatment with most antidepressants including the selective serotonin reuptake inhibitors (SSRIs), tricyclics (TCAs), and monoamine oxidase inhibitors (MAOIs) as well as acutely to some benzodiazepines such as alprazolam and clonazepam (Sheehan 1999).

Obsessive-compulsive disorder

The hallmark symptoms of obsessive compulsive disorder (OCD) are obsessions, which are recurrent, intrusive, and generally distressing thoughts, images or feelings. These obsessions are usually followed by compulsions, which are repetitive, ritualistic behaviors aimed to alleviate

obsessions (APA 1995; Foa et al. 1995). Occasionally, a patient may have only compulsions or obsessions, but most patients have both symptoms. To meet the criteria for OCD, obsessions or compulsions must occupy at least 1 h per day and cause significant distress or disability (APA 1995). This disorder affects about 2% of males and females equally. It usually begins during early adolescence or young adulthood but can also occur during childhood (Karno et al. 1988; Rasmussen and Eisen 1992). Like panic disorder, OCD is often comorbid with depression. Symptoms of this disorder are usually managed by cognitive behavioral therapies and treatment with SSRIs (for reviews see Ballenger 1999; McDougle 1999).

Social phobia

Individuals with this disorder report excessive fear of negative evaluation by others. These patients experience extreme discomfort in situations where they feel people are watching them or evaluating their performance. Therefore, they avoid social situations and may experience panic attacks when confronted with such situations (APA 1995; Mannuzza et al. 1995). Social phobia occurs more often in women although men are more likely to seek treatment (Kessler et al. 1994). Age of onset is usually between late childhood and adolescence. Social phobia has significant comorbidity with depression and alcohol abuse (Kessler et al. 1997). Patients suffering from this disorder respond well to chronic treatment with SSRIs and MAOIs or acute treatment with benzodiazepines such as clonazepam and with behavioral therapies (for review see Ballenger 1999).

Post-traumatic stress disorder

This condition develops subsequent to experiencing or witnessing a traumatic event. The precipitating event is a life-threatening assault that leads to lasting (at least 4 weeks) symptoms that include re-experiencing the trauma (e.g., flash-backs, memories, nightmares), avoidance of trauma-associated situations, numbing of emotions, and generalized arousal that fails to habituate (Brett et al. 1988; APA 1995). Women are twice as likely to develop this disorder as men and the prevalence is between 7 and 8% in the general population (Kessler et al. 1994). Cognitive behavioral therapy (Foa et al. 1999) and pharmacotherapy with antidepressants including SSRIs (Brady et al. 2000; Hidalgo and Davidson 2000a), MAO inhibitors, or tricyclic antidepressants (Kosten et al. 1991) are reported to be beneficial (for review see Ballenger 1999).

Generalized anxiety disorder

This condition is characterized by excessive and uncontrollable worries about life events. Approximately 3–4% of the population suffers from symptoms of this disorder in any year (Brown et al. 1994; APA 1995; Wolk et al. 1996). The disorder affects women more than men, may occur at any age from childhood through adulthood, and appears to have a familial association (APA 1995). By definition, generalized anxiety disorder has a minimum duration of 6 months but the pattern of excessive worrying is typically more enduring. The core symptom of worry is accompanied by symptoms of motor tension, autonomic reactivity or hypervigilance (APA 1995). This disorder has a high degree of comorbidity with depression and other mood disorders, both in its clinical presentation as well as its genetic pattern (Kendler 1996). Patients with this disorder respond well acutely to benzodiazepines and to a limited extent to chronic antidepressant treatments (for review see Ballenger 1999).

Relationship between animal models and clinical conditions

This brief description of DSM IV categories of anxiety disorders reveals differences not only in the symptoms, but also in age of onset, prevalence in males and females, and treatment response. For example, panic, post-traumatic stress disorder (PTSD), and generalized anxiety disorder are more prevalent in females. Benzodiazepines are more effective in treating generalized anxiety disorder, while antidepressants such as SSRIs are used more often to treat other anxiety disorders. While animal models of susceptibility or measures intended to reflect anxiety in animals often do not attempt to distinguish between these anxiety disorders, this is clearly an important consideration. For example, the sensitivity of punished responding in rodents to benzodiazepine anxiolytics might indicate that this test pulses brain systems contributing to the core symptoms of generalized anxiety disorder and thus, may be most useful for identifying potential treatments for this disorder (Barrett and Vanover 1993). Models of the fearful symptoms induced by conflict or threat are a common thread across anxiety disorders and the majority of anxiety models relate to this face validity component of anxiety (Table 1). However, very few other specific anxiety disorder diagnoses (e.g., PTSD) or unique symptoms are addressed in these models. An expansion of models to include novel dimensions or measures related to anxiety may lead to novel therapeutics for those symptoms not adequately treated and for developing a broader understanding of the neurobiological systems regulating fear and anxiety.

Strategies for developing novel models relevant to anxiety disorders

Most behavioral paradigms used as indices of fear or anxiety including conflict and exploratory tests (i.e., novel object or open field), examine the responses of normal animals in stressful situations. However, the clinical literature clearly indicates that exposure to

stressful or a traumatic event is only one of several factors determining the incidence of anxiety disorders (Hidalgo and Davidson 2000b). One way of investigating the possible etiology or molecules implicated in the neuropathology in animal models has been to manipulate presumed risk factors contributing to disorders and measure their impact on behavioral measures related to anxiety and fearfulness. Models that emulate predisposing environmental events, such as early life stress or adult trauma, have been useful for identifying brain circuits that are sensitized by exposure to adverse experiences (e.g., Graham et al. 1999). Similarly, genetic manipulations are employed to identify molecules contributing to anxious behavior (Heisler and Tecott 2000; Sibille et al. 2000; Weiss et al. 2000). These manipulations are particularly important for identifying possible targets for therapeutic development where few selective ligands exist.

The approach of mapping circuits and signaling pathways regulating the expression of specific anxiety-related behaviors holds distinct promise for the development of symptom-targeted compounds with novel mechanisms of action. This includes models of processes such as fear conditioning that are relevant across anxiety disorders (e.g., Fendt and Fanselow 1999) as well as identifying novel behavioral indicators related to general aspects of anxiety such as conflict or risk assessment behaviors (e.g., Blanchard RJ et al. 1993; Blanchard DC et al. 1998), or developing specific behavioral and biochemical indicators that may also be measured in patients. The following sections provide specific examples of these and other approaches that are attempting to extend animal models in a way that leads to new insights into etiology, basic processes, and differential treatment related to specific anxiety symptom presentation.

Modeling developmental risk factors

Early developmental factors have profound effects on adult resilience to stress and the incidence of mental disorders (DeBellis et al. 1999; Graham et al. 1999). For example, children who are victims of severe abuse or neglect have much higher risk of developing psychopathology and other chronic medical conditions (Herman et al. 1988; Seckl and Meaney 1993; Heim et al. 2000; Ladd et al. 2000). Early life stressors including emotional neglect and family strife (Ammerman et al. 1986; Trickett and McBride-Chang 1995) that are less extreme than abuse or neglect may also affect emotional and cognitive development. For example, longitudinal studies have shown that perception of parents as emotionally cold and distant increases the risk of chronic illnesses in adulthood by as much as four-fold compared to normal parental relationships (Russek and Schwartz 1997). This risk is seen equally for somatic illnesses such as hypertension and diabetes as well as emotional disorders such as depression and drug abuse. Such findings highlight the profound importance of early life experiences in the etiology of adult psychopathology.

Several model systems have been employed to evaluate specific factors contributing to increased or decreased susceptibility to adverse effects of stress and to elucidate the brain processes and neuroendocrine changes mediating these effects in animals (Rosenblum and Paully 1984; Coplan et al. 1992; Levine 1994; Meaney et al. 1996; Graham et al. 1999). In a variation of the rat maternal separation model, Meaney and colleagues have found that natural rates of licking and grooming of rat pups by mothers or changes in these behavioral measures induced by experimental manipulation affect expression of stress responses of pups in adulthood (Francis et al. 1999). Offspring of high and low grooming mothers were cross-fostered to investigate the complex interactions of early environment and genes in determining adult stress responses. Results of these studies revealed that qualitative differences in maternal behavior, in the absence of effects on total nursing time or pup growth, profoundly and permanently affected several measures of stress responses in the offspring. Offspring of low-grooming mothers showed significantly greater fearfulness in novel situations compared to the high-grooming cohorts (Caldji et al. 1998). The adult offspring of the high-grooming mothers showed a significantly greater benzodiazepine receptor density in the amygdala and increased noradrenergic alpha-2 receptor density in the locus coeruleus.

Early maternal environmental manipulation also has profound effects on hypothalamic pituitary adrenal (HPA) axis function. Adult offspring of low-grooming mothers demonstrated significantly greater ACTH and corticosterone responses during a 20-min restraint test compared to the offspring of high-grooming mothers (Liu et al. 1997). Levels of corticotropin releasing hormone (CRH) mRNA, and CRH receptor density were lower in offspring of the high grooming mothers (Caldji et al. 1998). Increases in glucocorticoid but not mineralocorticoid receptor mRNA have also been observed in the hippocampus of rats from the high-grooming group. These data suggest a permanent change in the corticosterone negative feedback loop of the HPA axis. More recent results suggest that these developmentally determined effects on adult HPA axis regulation may be mediated, in part, by modulatory effects on glucocorticoid receptor gene activation in the hippocampus (McCormick et al. 2000; Meaney et al. 2000). Such mechanisms may be of critical importance in the early stages of development and may affect protein expression throughout life.

Studies of offspring of low-grooming mothers have also revealed increased levels of extracellular dopamine, reduction in the density of the dopamine transporter, and greater behavioral, neurochemical and sensitization responses to cocaine administration (Meaney, personal communication). Such data suggest that early life experiences may also increase the risk of substance abuse and addiction in adulthood. In sum, many studies employing diverse paradigms in different species have revealed profound effects of early experience in determining adult

behavioral, hormonal, and neurochemical responses to stressors. In agreement with findings from clinical studies, these results clearly suggest that adverse early life experiences are risk factors for anxiety disorders and other mental and physical illnesses. These studies have the further potential for identifying the genetic, neurochemical, and experiential factors that account for individual differences in the separate or comorbid behavioral manifestations of early adverse experiences including symptoms of anxiety, depression, or other mental disorders and substance abuse.

Modeling genetic risk factors

Understanding the genetic factors contributing to disease vulnerability, course, or prognosis is an important step toward understanding pathophysiology. Studies of the physiological and behavioral consequences of targeted or random (Tarantino and Bucan 2000) mutation of genes implicated in the etiology or treatment of mental disorders represents an important complement to human genetics approaches.

Serotonin (5-HT) is an important neurotransmitter implicated in many neuropsychiatric disorders. Many psychiatric drugs including atypical antipsychotics, antidepressants, anxiolytics, and anorectic agents interact with 5-HT receptors. There are more than a dozen pharmacologically distinct serotonin receptor subtypes mediating a wide range of effects in different brain areas and in the periphery (for review, see Barnes and Sharp 1999). Development of receptor subtype-selective ligands and availability of receptor gene deletion mutants are proving highly useful for deciphering the roles of individual 5-HT receptor subtypes in mediating behaviors and the effects of psychotherapeutics (Tye et al. 1979; Broekkamp et al., 1989; Murphy et al. 1999; Bonasera and Tecott 2000).

Pharmacological and neuroanatomical evidence has implicated the $5HT_{1A}$ and $5HT_2$ receptors in the regulation of many behaviors used as indices of anxiety (Barrett and Vanover 1993; Griebel 1995). The $5HT_{1A}$ receptors are the principal somatodendritic autoreceptors that regulate the activity of serotonin neurons. They are also located postsynaptically in many brain areas (Barnes and Sharp 1999). The anxiolytic drug buspirone acts as a partial agonist at these receptors (Traber and Glaser 1987). The $5HT_{2C}$ receptor has been implicated in a wide variety of CNS functions including anxiety, appetite regulation, stress hormone release, locomotion, and thermoregulation (for review, see Koek et al. 1992). Results of recent studies employing mutant mice with targeted deletions of the $5HT_{1A}$ and the $5HT_{2C}$-receptor genes further support a role for these receptors in behavioral indices of anxiety (Heisler et al. 1998; Parks et al. 1998; Ramboz et al. 1998; Sibille et al. 2000).

Tecott and colleagues report that the development, weight gain, and home cage behavior of mice lacking $5HT_{1A}$ receptors appear normal during the first 6 months of life as compared with wild type controls (Heisler et al. 1998). However, when placed in the open field test, these animals showed significant thigmotaxis, a propensity to spend more time along the walls and away from the central areas of the field, suggesting that they may have greater fear and avoidance behavior. In the elevated zero maze, the $5HT_{1A}$ receptor null mutant mice demonstrated reduced time in the open quadrants, reduced distance traversed in the open segments, and fewer open quadrant entries and head dips. They also exhibited decreased exploration and increased avoidance of a novel stimulus presented in a familiar environment. These convergent results are consistent with a decrease in exploration associated with increased anxiety in $5HT_{1A}$ receptor knockout mice compared to the wild types. When gender differences were studied, the behavior of male mice was more disrupted than that of females.

$5\text{-}HT_{1A}$ receptor null mutant lines have been independently generated in three laboratories resulting in lines of mice in which the $5\text{-}HT_{1A}$ receptor mutation was bred to C57BL/6 (Heisler et al. 1998), 129/Sz (Ramboz et al. 1998), and outbred Swiss-Webster (Parks et al. 1998) genetic backgrounds. Given the inter-laboratory variability that may occur in behavioral studies of genetically modified mice (Crabbe et al. 1999), it is significant that concordant findings were reported in all three laboratories and across three background strains of $5\text{-}HT_{1A}$ receptor null mutants.

These studies exemplify the value of employing transgenic animals in studies of neuropsychiatric disorders. They also demonstrate the importance of careful selection of behavioral indices for assessing genetic contributions to complex behaviors. Previous studies have shown that subject and other experimental variations have profound effects on the degree to which 5-HT ligands affect behavioral measures of exploration or anxiety (Griebel 1995; Hogg 1996). Thus, in agreement with previous pharmacological study results, strain differences in reactivity and performance in behavioral tests (Gerlai 1996; Crawley et al. 1997) are essential considerations for interpreting behavioral effects of genetic mutations. These results also demonstrate that no single behavioral test or measure will reliably detect gene mutations that are related to anxiety or other mental disorders (Tarantino and Bucan 2000). A carefully selected test battery will help determine whether a given genetic manipulation selectively affects anxiety over more general behaviors such as activity level. Finally, the possibility that behavioral consequences of genetic manipulations may be due to indirect developmental effects highlights the need for combining other approaches with targeted and random genetic mutagenesis techniques to identify novel drug targets.

Modeling basic processes related to anxiety – fear-potentiated startle

Detailed and well-controlled neurobiological studies of basic processes can provide information that is relevant

for many mental disorders. For example, much of the basic understanding of brain mechanisms involved in the development and maintenance of fear and anxiety in animals has emerged from systematic studies employing conditioned fear or closely related paradigms (Kapp et al. 1990; Fanselow 1994; LeDoux 1996; Davis et al. 1997). Davis and colleagues (Davis et al. 1993) have utilized the fear-potentiated startle test to study fear circuitry. In brief, the paradigm involves placing a rat in a cage equipped to measure the amplitude of startle responses elicited by a loud burst of noise, either in the presence or absence of a light previously paired with electric shock. Animals previously exposed to the light paired with shock show a greater startle response to the noise in the presence of the light than in its absence, and this difference in startle amplitude is taken as an operational measure of fear. The brainstem pathways mediating the basic startle reflex have been identified (Lee et al. 1996). The association of the light with the shock is thought to occur in the BLA (Gewirtz and Davis 1997) and might involve a form of long-term potentiation (Fendt and Fanselow 1999). The plasticity that facilitates and imprints fear responses in the amygdala or related structures could be an important factor in variety of anxiety disorders, particularly those following severe aversive experiences such as PTSD. These changes are remarkably stable, consistent with the chronic and recurring nature of expressed fear in anxiety disorders.

A major clinical problem involves the inability of certain patients to overcome excessive fear or anxiety, even when any real danger has long subsided. Such patients have failed to extinguish fear memories or suppress irrational anxiety. In contrast to acquisition of conditioned fear, much less is known about the processes or brain regions involved in the extinction of fear responses. Fear memories are probably never permanently erased. Instead, new memories are formed that compete with or suppress the old fear memories. These processes may also be modeled. For example, in the rat, repetitive exposure to a conditioned fear stimulus in the absence of shock leads to a decrease in conditioned fear measured in that context (extinction). However, the fear memory can return, sometimes fully, when testing occurs in a different context (Bouton and King 1983). Furthermore, in a process called *reinstatement*, exposure to a novel stressor after extinction can lead to a return of conditioned fear. Even after 30 days of extinction, presentation of a single footshock fully restored fear-potentiated startle measured 24 h later (Gewirtz et al. 1997). These data support the hypothesis that extinction represents an active learning process and not the mere erasing of previous memories.

Another way to study brain systems involved in fear inhibition uses a procedure called *conditioned inhibition*. In this paradigm, animals are exposed on some trials to a light paired with shock and on other trials to a tone and the same light that signals the absence of shock. Using this procedure it is possible to measure fear-potentiated startle to the light and a reduction of fear-potentiated startle to the light in the presence of the tone (Falls and Davis 1997) in the same test session. This paradigm has significant experimental advantages because one can measure whether a certain lesion or drug interferes with fear inhibition versus fear elicitation. While lesions of the frontal cortex (Gewirtz 1997) or nucleus accumbens (Davis, personal communication) fail to block conditioned inhibition, data from some laboratories suggest a role for the hippocampus (Holland et al. 1999) or the lateral septum (Thomas 1988) in this important phenomenon. These and similar studies continue to be of great importance for understanding basic mechanisms of fear and anxiety and the processes regulating recovery from anxiety disorders.

Modeling characteristic phenomenology of anxiety disorders – panic response

Some unique symptoms that are strongly associated with mental disorders have been successfully and relatively unambiguously modeled in animals (e.g., Shekhar et al. 1996; Swerdlow et al. 1999). For example, physiological and behavioral components associated with panic responses such as changes in heart rate, respiration, blood pressure, conflict behavior, and social interaction have been modeled in studies attempting to identify neural and hormonal mediators of these responses (Shekhar 1994; Shekhar and Katner 1995; Shekhar et al. 1996). Results of these studies have suggested critical brain regions that may play a role in determining when and how this important survival reflex might transform into panic responses to minimal or inappropriate stimuli (Graeff et al. 1993). One such regulatory area is the dorsomedial hypothalamus (DMH). Blocking $GABA_A$ receptors in the DMH of rats results in a dramatic, panic-like state characterized by elevated heart rate, respiratory rate, and blood pressure and behavioral changes indicative of increased anxiety and fear (DiMicco et al. 1992; Shekhar 1994; Shekhar and Katner 1995). Blocking GABA transmission in the DMH also produces increased susceptibility to physiological arousal by lactate infusions resembling that seen in patients with panic disorder (Shekhar et al. 1996; Shekhar and Keim 1997). Circumventricular organs of the third ventricle, including the organum vasculosum lamina terminalis (OVLT) and subfornical organ, appear to be the sensory afferent sites that detect increases in plasma lactate levels and activate the DMH in this model (Shekhar and Keim 1997). Other results suggest that activation of norepinephrine, 5-HT, or NMDA type glutamate receptors also elicit panic-like responses in rats with decreased GABA transmission in the DMH (Shekhar and Keim 1997).

The anterior basolateral amygdala (aBLA) has also been implicated in the regulation of panic responses (Sanders and Shekhar 1991, 1995; Sajdyk and Shekhar 1997). Blockade of $GABA_A$ receptors in the aBLA with bicuculline methiodide has been shown to elicit panic-like responses such as increases in heart rate, blood pressure,

and respiratory rate, and increased anxiety as measured by the conflict, elevated plus-maze and social interaction tests (Sanders and Shekhar 1991; Sanders et al. 1995). In addition, priming the aBLA by repeated direct administration of subthreshold doses of bicuculline methiodide induced a long-lasting increase in susceptibility to experimentally induced anxiety responses to previously subthreshold stimuli (Sanders and Shekhar 1995). Repeated subthreshold activation of the CRF receptors in the BLA induced a unique priming phenomenon (Sajdyk et al. 1999) in which anxiety-like behavioral responses were primed but cardiovascular effects were not. Infusion of lactate or yohimbine in the bicuculline methiodide or CRF primed animals elicited significant and dramatic increases in heart rate and blood pressure and decreases in social interaction (Sajdyk and Shekhar 2000). Pretreatment with the anti-panic agent alprazolam before lactate infusions blocked these panic responses (Shekhar et al. 1999). These models have also identified the subfornical organ as a sensory relay nucleus for the lactate infusion stimulus to the BLA (Shekhar et al. 1999).

Behavioral models such as the panic model may help identify neural circuits, neurotransmitter systems, and mechanisms producing responses associated with anxiety disorders. Combining this targeted behavioral or phenomenological approach with models of genetic and developmental variation represents a promising direction for identifying critical neuroanatomical and neurochemical pathways relating to specific symptoms of anxiety disorders or unusual behavioral manifestations.

Modeling biochemical responses – HPA axis sensitization in PTSD

As indicated above, some neuropsychiatric disorders are associated with distinct biochemical responses to challenge tests. Examples include the greater sensitivity of panic disorder patients to the panicolytic effects of lactate infusion or the blunted dexamethasone (DEX) suppression of cortisol secretion in some depressed individuals (for review, see Holsboer 1999). In stark contrast with the suppression of cortisol release seen in depression, patients suffering from PTSD demonstrate an exaggerated inhibition of cortisol secretion following exogenous glucocorticoid administration. This finding suggests that in PTSD, the HPA axis feedback is sensitized as opposed to the blunting or desensitization that occurs following chronic stress or in depression (Kellner and Yehuda 1999). This unique HPA response has been the focus of efforts to model the development of PTSD (Liberzon et al. 1997). Liberzon and colleagues have developed a rodent stress paradigm consisting of 1 day of sequential exposure of adult rats to a variety of stressors including restraint stress, forced swim exposure, and ether anesthesia. This single prolonged stress exposure is followed by a period of "no stress" lasting for 7 days. This interval appears to be necessary for the emergence of the experimental effects. After 7 or 14 days, the animals are treated with saline or corticosterone and then challenged with restraint stress exposure. Exposure to this stressor sequence paradigm results in HPA axis hypersensitivity to glucocorticoid negative feedback (Liberzon et al. 1999a). Animals also exhibit increased levels of glucocorticoid receptor mRNA expression in the hippocampus and decreased mineralocorticoid mRNA levels by 7 days post-stress (Liberzon et al. 1999a). In view of the role of the hippocampus in fast negative feedback, these changes might reflect processes responsible for HPA supersensitivity. Repeated exposure of the rats to the same stressors, once per day, over consecutive days after the initial prolonged stress, does not maintain HPA axis hypersensitivity or glucocorticoid or mineralocorticoid mRNA changes, suggesting that compensatory processes like habituation occur following repeated stressor exposure. Thus, very specific conditions are required to model this characteristic neurohormone response seen with PTSD. Understanding the cellular mechanisms of such responses would help elucidate the cellular basis responsible for the sensitization of stress responses occurring in some anxiety disorders and may lead to the development of novel behavioral or pharmacological treatments.

Naturally occurring anxiety syndromes in animals

There exists a rich clinical resource available in veterinary behavioral medicine that is focused on dogs as natural models of anxiety disorders. The work of Overall (Overall 1997) and colleagues suggests that dogs represent a unique resource for understanding the neurobiological and genetic factors that contribute to maladaptive behaviors. Dogs may exhibit objective physiological and behavioral responses that appear in their clinical presentation to be similar to separation anxiety, thunderstorm or noise phobia, OCD, PTSD, and anxiety or panic attacks. These anxiety disorders are clinically diagnosed in the canine subjects and the FDA recently approved the use of pharmacological treatments of dogs for some anxiety disorders based on diagnosis (Overall 1994; King et al. 2000). Thus, a dog diagnosed with separation anxiety exhibits physical or behavioral signs of distress when denied access to their owner and shows significant improvement with clomipramine treatment (King et al. 2000). Noise phobia is diagnosed when dogs demonstrate a sudden, profound or extreme response to noise, manifested as escape behavior, avoidance, or physiological arousal.

The expression of behavioral indices of anxiety has been associated with the line or breed of dog and may be mapped over generations of family members. For example, anxiety-like behaviors are common in genetic lines of "shy" huskies or "nervous" pointers (Murphree et al. 1977; Overall et al. 1999). The affected individuals in these groups have distinctive anxiety "attacks" when approached by an unfamiliar observer. The response is characterized by increased heart rate, respiration, blunted cortisol response, and behavioral withdrawal.

This behavioral response also has a typical age of onset for the different breeds. The shy-huskies develop the disorder by 18–24 months of age while behavioral abnormalities develop in the nervous-pointers by 12–18 months. A diagnosis of compulsive behavior (Overall 1994) is considered when an animal displays inappropriate, repetitive, stereotypic behaviors that interfere with the animal's ability to function. Breeds that are more susceptible to these conditions include Great Danes, German Shepherds, and terriers. The recent discovery that hypocretin receptor mutations are responsible for narcolepsy behavior in selected colonies of Labradors and Beagles (Lin et al. 1999) is a clear example of the potential of these natural models of anxiety behavior for identifying critical genes regulating such behaviors.

Modeling effects of gender and age

One striking aspect of most anxiety disorders is the higher incidence in females as compared with males (e.g., Breslau et al 1995, DSM-IV). Gender also affects the clinical characteristics of anxiety disorders as well as the course, relapse rate, and the influence of early childhood vulnerability factors on these parameters (Pigott 1999). For example, two early childhood vulnerability factors for adult anxiety disorders, behavioral inhibition and separation anxiety disorder, are more prevalent and enduring in girls than boys. Similarly, the focal symptom of generalized anxiety disorder, worry, is far more prevalent in women and girls than in men and boys across cultures and ethnic backgrounds (Blanchard 1998). The age of onset of several anxiety disorders including panic, generalized anxiety, and phobias is earlier in females than in males. Complications of anxiety disorders such as agoraphobia and depression are much more frequent in females (Pigott 1999). Shear (1997) found that quality of life is more severely disrupted in females than in males on a variety of measures of symptom severity, including effects on interpersonal relationships and functional impairment. Little is known about the impact of gender in treatment outcome but recent studies have demonstrated a greater relapse rate following remission of panic disorder in female subjects (Yonkers et al. 1998). Sex differences in psychotropic drug metabolism and clearance alone can have direct effects on the efficacy of these treatments for mental disorders in women (Pigott 1999).

These clinical observations suggest that gender impacts multiple points in the illness pathways leading to anxiety disorders including genetic, developmental, hormonal, neurochemical, and psychosocial processes. For example, prospective data suggest that being female is itself a risk factor for PTSD (Breslau et al. 1995). However, epidemiological data also suggest that women are at higher risk of exposure to types of traumatic events that increase the probability of developing PTSD in both males and females (Hidalgo and Davidson 2000b). Thus, biological, hormonal, and cultural factors may contribute to sex differences in some disorders. Basic research studies in animals may help to determine the degree to which these differences are mediated by differences in brain physiology.

Given the preponderance of sex differences in so many aspects of anxiety disorders, it is surprising to find how few basic animal studies have considered gender in developing or employing models to study the neurobiology of anxiety. A recent survey of the animal studies of serotonin and anxiety related behaviors revealed that approximately 90% utilized males exclusively (Blanchard et al 1995). Clearly, this major deficiency has delayed progress towards understanding the processes contributing to anxiety disorders and most likely deterred the development of gender-specific treatments.

Male and female laboratory rats show profound sex differences in performance in exploratory tests (Archer 1975; Norton 1977; Johnston and File 1991) and in defensive response to threat. When the threat stimulus is a potential danger, as opposed to an actual predator, the major defensive behavior is risk assessment, measured by orientation to the threat stimulus (e.g. a cat odor) and the assumption of a specific "stretched" posture, sensory scanning (sniffing, visual scanning), and approach/investigation of the stimulus (Blanchard 1997). Such activities have been shown to reflect an information-gathering mode in which the animal may assess the threat potential of a stimulus (Pinel et al. 1989). Both risk assessment and defensive threat/attack behaviors are modified by anxiolytic drugs (Blanchard RJ et al 1993; Blanchard DC et al. 1998; Griebel et al. 1995; 1996). Females exhibit increased incidence or duration of risk assessment and behavioral inhibition (Blanchard et al. 1991); alarm vocalizations (Blanchard et al. 1992) and more frequent unprovoked defensive attacks at a predator (Blanchard et al. 1980). Females display less freezing behavior compared to males (Blanchard et al. 1991). In conjunction with findings of estrus cycle effects on other threat- or stress-linked behaviors (Fernandez-Guasti and Picazo 1992; Haney and Miczek 1993; Paré and Redei 1993; Frye et al. 2000), these phenomena suggest that gonadal hormones may serve as neuromodulators for the brain systems underlying defense, and that disregulation of these same neural and hormonal systems may be involved in some anxiety disorders. Thus, novel drug screening that includes both male and female subjects may reveal gender specific pharmacotherapeutic effects.

Neuroanatomical models – insights from human neuroimaging

Neuroimaging has considerable potential for revolutionizing the study of human neuropsychiatric disorders. Results of structural and functional imaging studies using psychiatric patients are just beginning to identify neuroanatomical systems and neurotransmitters that are disrupted in mental disorders. Similarly, imaging studies in normal subjects continue to be essential for identifying

brain regions where activity is modified during presentation of fearful stimuli and the factors that modify patterns of activity. For example, results of a recent functional imaging study examining amygdala activation during exposure to neutral or fearful faces revealed greater activation of amygdala in normal adults during fearful face exposures. In contrast, children demonstrated amygdala activation to both neutral and fearful faces. This result is consistent with the observation that children may have a lesser ability to distinguish these stimuli (Thomas et al. 2001). Amygdala activation of male but not female children was decreased with repeated face presentations. These and other results clearly indicate that age and gender are important factors in determining brain fearful and anxious responses and are consistent with epidemiological data on anxiety disorders. The use of longitudinal or cross-age studies of brain activity during performance of specific tasks or ligand binding studies might help to identify brain systems that are disrupted within specific stages of development, particularly in adolescence (Thomas et al. 2001). Eventually, such approaches may be used to identify individuals atrisk for developing disorders.

Unique opportunities exist in neuroimaging studies of clinical populations demonstrating specific anxiety symptoms (Dager et al. 1996; Davidson et al. 1999). For example, imaging results have implicated the orbitofrontal cortex-striatal pathways in OCD (Baxter et al. 1987; Saxena et al. 1999) and amygdala and anterior cingulate cortex in PTSD (Liberzon et al. 1999b; Rauch et al. 2000). Some brain regions are consistently activated across several anxiety disorders. For example, symptom provocation paradigms have consistently been shown to activate the amygdala and the right side of the prefrontal cortex across anxiety patient populations (Davidson et al. 1999). These identified brain regions could also be examined for changes in activation in animal models emulating aspects of anxiety. In addition, new models could be developed to replicate these specific activational defects using parallel procedures and measures.

Full realization of the potential value of neuroimaging will require technological improvements in spatial and temporal resolution and standardization of data processing across laboratories and patient groups. Improvements in the accuracy of diagnoses will further increase the likelihood of identifying brain regions selectively affected in anxiety and other mental disorders. These improvements will be essential to develop neuroimaging as a diagnostic for mental disorders and for developing treatment strategies aimed at normalizing specific brain deficits. Animal models will clearly play an important role in fostering these developments.

Considerations for improving anxiety disorder models

Many of the behavioral measures used to assess aspects of anxiety in animals rely on conflict paradigms that incorporate measures of innate or learned responses to aversive situations in normal animals. These tests have been extremely useful as initial screens for identifying manipulations or drugs affecting anxiety. However, the components of anxiety assessed by these models remain poorly defined. Results of conflict tests represent combined effects of manipulations on approach (exploration) and avoidance or fearfulness. As recently shown by Dulawa et al. (1999), one can examine the relative contributions of these factors by employing multiple tests in which behavioral measures reflect predominantly approach and avoidance responses. In that study, deletion of the dopamine D_4 receptor had a greater effect on emergence and novel object test measures and less effect on open field activity, suggesting that this deletion induced greater deficits in exploration than fearfulness (Dulawa et al. 1999).

Finally, results of several studies have clearly shown that conflict test measures, like other behaviors, are affected by minor changes in the testing apparatus, as well as by species, strain, and sex of the subjects (Barrett and Vanover 1993; Griebel 1995; Hogg 1996). Minimally, these data indicate that no single measure or model is sufficient to identify anxiety in animals. Additional basic studies are needed to identify the extent to which behavioral measures reflect changes in neurobiological systems regulating anxiety and fear.

Nearly half of patients suffering from anxiety disorders also suffer from depression (Clark 1989). There is also a high degree of comorbidity of anxiety with other medical conditions, such as irritable bowel syndrome, alcohol and drug abuse, and cardiovascular disorders (Kessler et al. 1994; Kendler 1996). Susceptibility models that examine the impact of early life stressors or genetic variability on the development of stress responses in adulthood are important for developing an understanding of the etiology of anxiety and other mental disorders. These risk factor models may also provide essential clues for unraveling the determinants of comorbidity of anxiety diagnoses with other disorders such as depression or substance abuse. Essential future studies will identify individual differences that determine the impact of early life events and genetic variation on expression of specific phenotypes associated with anxiety and other mental disorders.

Conditioned fear is a highly relevant paradigm for understanding the neurological underpinnings of anxiety symptoms common across subclassifications. The detailed descriptions of neurocircuits mediating the acquisition and maintenance of conditioned fear responding highlight brain regions where neurochemical changes are likely to provide leads for focused therapeutic development. Additional directions for therapeutic development might be found with expansion of cognitive models exploring mechanisms responsible for the extinction of conditioned fears and the persistence of maladaptive behaviors subsequent to conditioned fear acquisition. Comparative human and animal neuroimaging studies across the lifespan and for both sexes will most likely be important for identifying critical brain circuits regulating

normal and pathological fear. Results of these studies will be useful for focused pharmacological and cognitive therapeutic development.

The brief review of the distinct syndromes of anxiety disorders (APA 1995) each with unique behavioral, epidemiological, and treatment response profiles suggests that different neurobiological mechanisms are affected for the different anxiety disorder subtypes (Martin 1998). Some distinct opportunities exist for modeling behaviors, physiology, or neuroendocrine responses unique to individual anxiety syndromes. Models of panic, neuroendocrine patterns in PTSD, and unique behaviors associated with OCD appear particularly promising as targets for neurobiological studies aimed at discovering new targets for therapeutic development. Finally, naturalistic models such as nervous dogs may be important for determining the genetic susceptibility of discrete behaviors.

Both clinical and basic studies have revealed important sex differences in the expression of anxiety disorders as well as the behavioral expression and possibly the acquisition of fear-motivated behaviors. Studies examining the impact of genetic and environmental factors on basic neurobiological phenomenon such as fear conditioning as well as the onset, chronicity, behavioral expression, and treatment response will be useful for determining the neurobiological bases of some of these sex differences.

Conclusions

Human neuropsychiatric disorders develop as a consequence of genetic/developmental predispositions that affect sensitivity to life stressors and the initiation of pathophysiological processes. The probability of developing comprehensive animal models that accurately reflect the relative influences of contributing factors is probably quite low. However, as clearly outlined in this workshop, ample opportunity remains to better define and extend existing models and to develop new models that consider the impact of combined factors in determining anxious behaviors. Clinical neuroimaging will extend the clinical description of the disorder and point to new avenues for model development. By emulating one or more aspects relevant to anxiety disorders, models may identify systems mediating defined behavioral endpoints and develop more effective treatments.

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